



## RESEARCH ARTICLE

# Evaluation of *in vitro* Cholesterol esterase inhibitory and *in vivo* Anti-hyperlipidemic activity of aqueous extract of *Plukenetia conophora* Mull. Arg. (Euphorbiaceae)

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**Abstract**

Hyperlipidemia is a condition of abnormally high lipids levels in the blood which has been ranked as one of the greatest risk factors contributing to prevalence and severity of coronary heart disease. The available antihyperlipidemic drugs have been associated with some side effects however, herbal management of hyperlipidemia are relatively safe, cheap and readily available. *P. conophora* is an edible plant consumed in Nigeria as snack and speculated to have beneficial effect on blood lipid profile. The present study evaluates anti-hyperlipidemic effect of aqueous extract of cooked *P. conophora* nut using *in vivo* and *in vitro* experimental models.

The anti-hyperlipidemic activity was evaluated using tyloxapol induced-hyperlipidemic rats by intraperitoneal injections of Tyloxapol at a dose of 300 mg/kg body weight and high cholesterol-diet induced rats by oral administration of high cholesterol diet for 60 days. Cholesterol esterase enzyme inhibition was used for the *in vitro* evaluation.

Aqueous extract of *P. conophora* at varying doses, reduced the elevated lipid parameters in both models; the dose of 500 mg/kg showed comparable hypolipidemic effects with standard drug (Simvastatin) at 10 mg/kg ( $P < 0.01$ ). The extract also inhibited cholesterol esterase enzyme with  $IC_{50}$  value of  $129.30 \pm 0.10 \mu\text{g/ml}$  while Simvastatin with  $IC_{50}$  value of  $51.42 \pm 0.13 \mu\text{g/ml}$ . Preliminary phytochemical analysis revealed the presence of; Flavonoids, saponin, cardiac glycoside, alkaloids, tannins, steroids and reducing sugar.

*P. conophora* extract exhibited strong hypolipidemic activity and the dose of 500mg/kg demonstrated equipotent activity as the standard drug; Simvastatin 10mg/kg. The extract also showed inhibitory activity against pancreatic cholesterol esterase enzyme; hence can be used to limit absorption of dietary cholesterol, prevent and treat hyperlipidemia.

**Keywords:** pancreatic cholesterol esterase; Tyloxapol; High Cholesterol diet; Plukenetia conophora; Simvastatin

## Introduction

Hyperlipidemia is an asymptomatic condition of abnormally elevated levels of any or all lipids and/or lipoprotein in the blood

and is a highly predictive risk factor for atherosclerosis, coronary artery diseases and cerebral vascular diseases [1]. Hyperlipidemia is a global pandemic and a major risk factor for cardiovascular disease. Cardiovascular diseases and atherosclerosis are the most common causes of mortality and morbidity worldwide; an approximately 12 million people reportedly die of cardiovascular disease each year worldwide [2]. The burden of the

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disease in terms of morbidity, mortality, and medical costs is immense. It is a leading cause of death for both men and women of all races and ethnicities worldwide [3]. The prevalence of hyperlipidemia has dramatically increased worldwide due to modern lifestyle and increase consumption of high fat diet [4].

The modern pharmacological therapy for the management of hyperlipidemia is costly and associated with multiple side effects resulting in patient non-compliance and therapeutic failure. Thus, there is a need to explore alternative therapies particularly from plant sources as these are cost effective and possess minimal side effects [5]. Medicinal plants play a major role as hypolipidemic agent; hence natural products are now part of the current therapy for hyperlipidemia. A number of medicinal plants have shown their beneficial effect on cardiovascular disease by virtue of their lipid lowering effect [6]. In recent times, edible plants have increasingly become attractive alternatives to prevent or treat various types of diseases because of easy availability and easy absorption in the body [7].

Nigerian walnut ( *Plukenetia conophora* ) is an edible nut widely consumed as snack in Nigeria based on speculations that these plant products after consumption has beneficial effect on blood lipid profile but there is little scientific proof to the effect. It belongs to the Euphorbiaceae Family and in Nigeria; it has various vernacular names such as asala or awusa (Yoruba), ukpa (Ibo), oke okpokirinya (Imo) and okhue or okwe (Edo) [8, 9]. *P. conophora* leaves are used traditionally for curing headache and as remedy against cancer, sexual impotence, inflammation, antitussive, coronary heart disease [10]. The fresh nuts are used for curing snakebites and as tonic and aphrodisiac [8].

This present work is aimed at evaluating the effect of cooked Nigerian walnuts ( *P. conophora* ) aqueous extract on lipid profile in hyperlipidemic-induced rat models ( *in vivo* ) and *in vitro* cholesterol esterase inhibitory analysis.

## Materials and methods

### Plant collection and Preparation of Plant Extract

*Plukenetia conophora* (Nigerian walnut) cooked nuts were purchased from Ketu market in Lagos state, Nigeria. The Plant material was authenticated at the Department of Botany, University of Lagos, Akoka, where a voucher specimen was deposited under LUH 6941. The cooked nuts were shelled, weighed and pulverized with an electric blender at medium to high speed using distilled water as solvent. It was filtered using muslin-drawstring bags and milk-like clear filtrate was obtained. The extract was freeze dried with Lyotrap ultra freeze dryer from LTE scientific to obtain a cream-colour semi solid oily residue, with percentage of 12.21%.

### Preliminary Phytochemical screening

Preliminary phytochemical screening of the aqueous extract of *P. conophora* for various. Phytochemical classes were carried out based on a modified version of the reported methods by Trease and Evans, 2006; Sofowora, 1993 [11, 12]. The extract was screened for the presence of saponins, flavonoids, tannins, phenols, steroids, terpenoids and cardiac glycosides, reducing sugar, alkaloids and anthraquinones.

### Drugs and chemicals

Tyloxapol (Triton W 1339), *p* -nitrophenylbutylrate (p -NPB), taurocholic acid sodium salt, porcine cholesterol esterase and sodium phosphate buffer of pH (7. 0) were purchased from Sigma-Aldrich Co (Bristol scientific Co, Lagos, Nigeria), Simvastatin 10mg (Teva Pharmaceutical, UK). All the other drugs and chemicals used in this study were commercially obtained and were of analytical grade.

## Experimental animals

Adult albino rats (Wistar strain) weighing between 90-120g were obtained from Kodak animal farm, Sango-Ota Ogun-state, Nigeria and were kept in the animal laboratory centre under appropriate temperature, humidity and light condition for the study. The animals were acclimatized for two weeks under normal environmental conditions with 12 hour light/dark cycle and sawdust for beddings. They were divided into eleven groups of six animals each and kept in separate cages. They were fed normal rat chow and water *ad libitum*. Rat weight was measured weekly with a laboratory electronic scale. The animal studies were performed in accordance with the ethical standards laid down by the Declaration of Helsinki and the guiding principles in the care and use of animals [13].

### *In vivo* experimental design

#### *Tyloxapol – induced hyperlipidemic model*

Tyloxapol – induced hyperlipidemic model reported by [14], was followed with slight modification. Animals were divided into five groups of six animals in each group (n = 6 per group). Blood was collected from all the animals by ocular puncture to obtain their baseline lipid profile. Hyperlipidemia was induced experimentally in 12 h-fasted rats by a single intraperitoneal injection of Tyloxapol (Triton WR-1339) 300 mg/kg body weight dissolved in 0.9% normal saline. Twenty four hours after administration of Tyloxapol, blood was collected from all the animals by ocular puncture. They were used for further investigation and treatment.

**High fat diet induced hyperlipidemic model** High fat diet- induced hyperlipidemic model was studied using the method described by [15] with slight modifications. The animals were divided into five groups of six animals in each group. Blood was collected from all the animals by ocular puncture to obtain their baseline lipid profile after overnight fasting. Hyperlipidemia was induced by given high fat diet (egg yolk in coconut oil) orally by gavage for 60 days. The high fat diet was prepared using half boiled egg yolk (50g) made into paste by meshing in a mortar and gradually adding coconut oil up to 100mls (0.5g/ml). The paste was prepared *in situ* and 1ml was administered to the animal daily using oral cannula for 60 days.

**Drug Treatment and Animal Groups** A concentration of 100mg/ml of the *P. conophora* aqueous extract was prepared by dissolving 2g (2000mg) of the extract in 19.6 mLs of distilled water using 2% tween 80 (0.4mls) as the suspending agent. A concentration of 2mg/ml was also prepared for the standard drug, simvastatin by dissolving 10mg of the powdered tablet in 5ml of distilled water. The eleventh group of rats (Group 6) for each of the experiment was the normal control, which received distilled water and normal rat chow throughout.

**Tyloxapol-induced hyperlipidemic rats were treated for a period of 7 days as follows :**

**Group 1:** received aqueous extract of *P. conophora* 125mg/kg body weight orally.

**Group 2:** received aqueous extract of *P. conophora* 250mg/kg body weight orally.

**Group 3:** received aqueous extract of *P. conophora* 500mg/kg body weight orally.

**Group 4:** received standard drug, simvastatin 10mg/kg body weight orally.

**Group 5:** served as negative control and received no drug treatment.

**Group 6:** served as normal control, received distilled water and normal rat chow throughout

**High-fat-diet- induced hyperlipidemic rats were treated for a period of 15 days as follows:**

**Group 1:** received aqueous extract of *P. conophora* 125mg/kg body weight orally.

**Group 2:** received aqueous extract of *P. conophora* 250mg/kg body weight orally.

**Group 3:** received aqueous extract of *P. conophora* 500mg/kg body weight orally.

**Group 4:** received standard drug, simvastatin 10mg/kg body weight orally.

**Group 5:** served as negative control and received no drug treatment

**Group 6:** served as normal control, received distilled water and normal rat chow throughout

**Biochemical assay** Blood was collected by ocular puncture in heparinized bottle and centrifuged at 3000 rpm for 10min at room temperature to get the serum. Serum total cholesterol, triglyceride, HDL-C and LDL-C was estimated using Automated Analyzer at the Central Research Laboratory (APIN Laboratory), Lagos university teaching hospital, Idi-araba, Lagos.

**In vitro Cholesterol esterase Enzyme Inhibitory Activity:** Pancreatic cholesterol esterase inhibitory activity of the aqueous extract of *P.conophora* was determined according to the method reported by [16].

The inhibitory activity was evaluated and performed in the presence of sodium taurocholate (TC) with p-nitrophenyl butyrate (pNPB) as chromogenic substrate. Stock solution of porcine cholesterol esterase (19.5ng/ml) and taurocholate (12mM) were prepared by using 100mM sodium phosphate buffer of pH (7. O). Stock solution of pNPB (200μM) and various concentrations (20, 40, 80 and 160μg/ml) of the aqueous extract of *P. conophora* and standard drug, simvastatin were prepared by using Acetonitrile (6%). A final volume of 1ml was taken into a cuvette containing 430μl of assay buffer, 500μl of TC solution, 40μl of acetonitrile, 10μl of pNPB solution and 10 μl of the inhibitor (extract or standard drug) solution were added and thoroughly mixed. The mixture was incubated for 2 min at 25°C and the reaction was initiated by adding 10μl of the enzyme solution. Uninhibited enzyme activity (Blank) was determined by adding acetonitrile instead of the inhibitor solution (extract or standard drug). Absorbance was measured at 405 nm. The percentage inhibition was calculated by using method of [17].

Cholesterol esterase inhibition percentage,  $I = [1 - a] \times 100$

a= enzyme activity with inhibitor /enzyme activity without inhibitor

## Statistical analysis

All data were expressed as means  $\pm$  standard error of mean (SEM) and Inhibitory concentration (IC<sub>50</sub> ) values were calculated from plots of log concentration of inhibitor versus percentage inhibition curves by using Graph pad prism version 5.0 software

The statistical parameter applied was student's t-test and one-way ANOVA (Analysis of variance) followed by Dunnett's post – hoc multiple comparison tests.

All the statistical analysis were performed using GraphPad Prism software version 5.0

Confidence limit was chosen at 95% ( $P < 0.05$ ), 99 % ( $P < 0.01$ ) and 99.99% ( $P < 0.001$ )  $P < 0.05$  = Significant  $P < 0.01$  = Very significant  $P < 0.001$  = extremely significant  $P > 0.05$  = Insignificant  $n = 6$

## Results and discussion

Hyperlipidemia has been documented as one of the major risk factors involved in the development of cardiovascular diseases. Evidence from lipid lowering trials has clearly established that reduction of total cholesterol or LDL-C is associated with a decreased risk of atherosclerosis and coronary heart disease [18]. High cost of synthetic antihyperlipidemic drugs and the lag on the desired properties of safety on long term use affect patient's compliance [19]. Hence, natural products have become the best alternative strategy for the development of safe, effective, affordable and acceptable antihyperlipidemic agent. A number of medicinal plants have shown their beneficial effect on cardiovascular disease by virtue of their lipid lowering effects [20].

The present study was designed to verify the claimed antihyperlipidemic activity and investigate the possible mode of action of the lipid-lowering effect of *P. conophora* nut using different models. Intraperitoneal injection of tyloxapol is known to block the uptake of lipoprotein from the blood circulation by extra hepatic tissues, resulting in an increase in the level of circulatory lipoproteins [21].

The induction of hyperlipidemia by Tyloxapol interperitoneal injection of 300mg/kg in this study proved to be effective in the animals, peaking 72 hours after application of the drug. A statistical significant increase ( $P < 0.01$ ) in LDL-C, Triglyceride and total cholesterol was observed compared to baseline in the induced animal and significant decrease ( $P < 0.05$ ) was observed in the HDL-C [22] stated that the large increase in plasma cholesterol and triglycerides due to Tyloxapol administration resulted mostly from an increase of VLDL secretion by the liver, accompanied by a strong reduction of VLDL and LDL-C catabolism.

Following the administration of varying doses of aqueous extract of *P. conophora* nut on the tyloxapol-induced hyperlipidemic rats for a period of 7days; the dose of 250mg/kg and 500mg/kg exhibited a significant decrease ( $P < 0.05$ ) in the elevated levels of serum total cholesterol and LDL-C as well as significant rise in the HDL-C, suggesting a beneficial modulatory influence on cholesterol metabolism and turnover. The dose of 500mg/kg of *P. conophora* aqueous extract displayed comparable hypolipidemic effect with the standard drug simvastatin

10mg/kg. The serum lipid level of normal control group, negative control group and treated groups are shown in table 2.

Feeding the animals with high cholesterol diet for 60 days resulted in significant elevation of serum TC, TG and LDL-C concentration ( $P < 0.01$ ) and decrease in HDL-C ( $P < 0.05$ ) compared to baseline measurement, this is in line with previous findings reported by [23]; who demonstrated that feeding Wistar rats a high cholesterol diet for 60 days induced hyperlipidemia. There was also significant increase in the body weights of rats on high fat diet compared to normal control group. The effect of *P. conophora* extract on the induced hyperlipidemic rats and the results are shown in Table 3. *P. conophora* dose of 250mg/kg significantly reduce TC and LDL-C ( $P < 0.01$ ) and increased HDL-C ( $P < 0.05$ ) while *P. conophora* dose of 500mg/kg significantly reduce TG, TC and LDL-C ( $P < 0.001$ ) and increased HDL-C ( $P < 0.05$ ) similarly as the standard drug Simvastatin 10mg/kg. Reduction of plasma total cholesterol in response to treatment with the aqueous extract of *P. conophora* was observed to be associated with the significant decrease of its LDL-C, which in fact, is the target of several hypolipidemic drugs. This result suggests that the cholesterol lowering activity of *P. conophora* could possibly be due to a rapid catabolism of LDL-C through its hepatic receptors for its final elimination in the form of bile acids [24].

In general, pancreatic cholesterol esterase plays an important role in hydrolyzing dietary cholesterol esters which liberates free cholesterol in the lumen of the small intestine [25]. It enhances the incorporation of cholesterol into mixed micelles and aids transport of free cholesterol to the enterocyte [26]. Inhibitors of Cholesterol esterase is expected to limit the bioavailability of dietary cholesterol derived from cholesterol esters and limit the absorption of free cholesterol.

The *in vitro* cholesterol esterase inhibitory activity of *P. conophora* aqueous extract was examined (Table 4) and the inhibitory effect of the extract against bovine pancreatic cholesterol esterase showed good activity comparable with that of the standard drug used, simvastatin. Extract exhibited good Cholesterol esterase activity ranging from 36.08 to 72.40% inhibition (for 20 to 160  $\mu\text{g/ml}$ ) with  $\text{IC}_{50}$  value of  $129.30 \pm 0.10 \mu\text{g/ml}$ . However, they were less potent in comparison to corresponding values of the standard, Simvastatin with  $\text{IC}_{50}$  value of  $51.42 \pm 0.13 \mu\text{g/ml}$ . Similar results have been reported by [10]; that safflower and senna extracts demonstrated a potent inhibitory activity against pancreatic cholesterol esterase with the  $\text{IC}_{50}$  values of  $(1.70 \pm 0.15)$  and  $(2.57 \pm 0.21) \text{ mg/mL}$  respectively.

The preliminary phytochemical investigations of the aqueous extract of *P. conophora* revealed the presence of alkaloids,





**Table 3 Effect of *P. conophora* extracts on Total Cholesterol, Triglycerides, LDL-C and HDL-C in High-Cholesterol-diet-model rats**

Groups	HDL-C (mmol/L)	LDL-C (mmol/L)	TC (mmol/L)	TG (mmol/L)
Group 1 (HCD + <i>P. conophora</i> 125mg/kg)	1.23 ± 0.09 <sup>ΔΔ</sup>	0.96 ± 0.05 <sup>**</sup>	4.06 ± 0.23 <sup>ΔΔ</sup>	1.76 ± 0.07 <sup>ΔΔ</sup>
Group 2 (HCD + <i>P. conophora</i> 250mg/kg)	1.25 ± 0.05 <sup>ΔΔ</sup>	1.15 ± 0.08 <sup>ΔΔ</sup>	3.62 ± 0.33 <sup>*</sup>	1.31 ± 0.08 <sup>**</sup>
Group 3 (HCD + <i>P. conophora</i> 500mg/kg)	1.24 ± 0.05 <sup>ΔΔ</sup>	0.89 ± 0.16 <sup>***</sup>	2.93 ± 0.03 <sup>***</sup>	1.41 ± 0.07 <sup>*</sup>
Group 4 (HCD + Simvastatin 10mg/kg)	1.37 ± 0.13 <sup>*</sup>	0.75 ± 0.11 <sup>***</sup>	2.93 ± 0.08 <sup>***</sup>	1.41 ± 0.20 <sup>*</sup>
Group 5 (HCD control)	0.98 ± 0.07 <sup>ΔΔ</sup>	1.54 ± 0.09 <sup>***</sup>	4.57 ± 0.17 <sup>***</sup>	1.90 ± 0.08 <sup>***</sup>
Group 6 (Normal control)	1.19 ± 0.07	0.34 ± 0.03	2.11 ± 0.06	1.15 ± 0.04

**Table 4 *In vitro* Pancreatic Cholesterol Esterase inhibition by aqueous extract of *P. conophora* cooked nut**

Conc (μg/ml)	<i>P. conophora</i> Inhibition (%)	IC <sub>50</sub> (μg/ml)	Simvastatin Inhibition (%)	IC <sub>50</sub> (μg/ml)
20	36.08 ± 0.54	129.30 ± 0.10	42.50 ± 0.40	
40	45.73 ± 0.58		59.92 ± 0.08	
80	57.17 ± 1.13		73.95 ± 1.47	51.42 ± 0.13
160	72.40 ± 0.03		90.31 ± 1.97	

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## Conflict of interest

The authors declare that there is no conflict of interest associated with this publication.

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## References

- [1] Amuamuta A. A review on risk factors/Indicators and effects Of Hyperlipidemia. Middle-east journal of Scientific Research. 2014;22(6):886–893.
- [2] Sivaelango G, Kumaran P, Kumaravel P, Revathi P, Jaswant A. Antihyperlipidaemic Activity of *Spermacoce hispida* Ethanolic Extract in Triton WR-1339 Induced Hyperlipidaemic Rats. Journal of Applied Pharmaceutical Science. 2012;2(2):95–98.
- [3] Tekes-Manova D, Israeli E, Shochat T, Swartzon M, Gordon S, Heruti R. The Prevalence of Reversible Cardiovascular Risk Factors in Israelis aged 25-55 years. Isreal Medical Association Journal. 2006;8:527–531.
- [4] Adisakwattana S, Moonrat J, Srichairat S, Chanasit C, Tirapongporn H, Chanathong B, et al. Lipid-Lowering mechanisms of grape seed extract (*Vitis vinifera* L) and its antihyperlipidemic activity. Journal of Medicinal Plants Research. 2010;4(20):6–18. Lipids in Health and disease.
- [5] Siddiqi HS, Mehmood MH, Rehman NU, Gilani AH. Studies on the antihypertensive and antidyslipidemic activities of *Viola odorata* leaves extract. Lipids in health and disease. 2012;11(1):6.
- [6] Pandit K, Karmarkar SM, Bhagwat AM. Evaluation of antihyperlipidemic activity of *Ficus hispida* linn leaves in triton wr-1339 (tyloxapol) induced hyperlipidemia in mice. Int J Pharm Pharm Sci. 2011;5(5):188–191.
- [7] Pandit K, Karmarkar S, Bhagwat A, Adisakwattana S, In-trawangso J, Hemrid A, et al. Extracts of edible plants inhibit pancreatic lipase, cholesterol esterase and cholesterol micellization, and bind bile acids. In: IPE, editor. Evaluation of Antihyperlipidemic Activity Of *Ficus hispida* Linn Leaves In Triton Wr-1339 (Tyloxapol). vol. 3. ASHS Press; 1999. p. 457–462. Perspective on new crops and the new uses.
- [8] Hutchinson J, Dalziel JM, et al. Flora of west tropical Africa. Flora of West Tropical Africa The British West African Colonies, British Cameroons, the French and Portuguese Colonies south of the Tropic of Cancer to Lake Chad, and Fernando Po. 1958;1(Part II).
- [9] Burkill H. Entry for *Plukenetia conophora*. In: The Useful Plants of West Tropical Africa. Kew, UK: Royal Botanic Gardens;. .
- [10] Iwu MW, Duncan AR, Okunji CO, et al. New antimicrobials of plant origin. Perspectives on new crops and new uses ASHS Press, Alexandria, VA. 1999;p. 457–462.
- [11] Evans WC, Trease E, Pharmacognosy. London; 2002. 15th edition W. R. Saunders.

- [12] Sofowora E. Medicinal Plants and Traditional Medicine in Africa. In: Spectrum books Ltd; 2008. p. 150–153. 3rd Edition.
- [13] Guiding principles for research involving animals and human beings. Am J Physiological Regul/Integr Comp physiol. 2002;283:281–283.
- [14] Tamer A, Mohamed M, Mohamed N, Dalia I. Antihypercholesterolemic Effects of Mushroom, Chrysin, Curcumin And Omega-3 in Experimental Hypercholesterolemic Rats. Journal of Food and Nutrition Research. 2015;3(2):77–87.
- [15] Dhulasavant V, Shinde S, Pawar M, Naikwade NS. Anti-hyperlipidemic activity of Cinnamomum tamala Nees. on high cholesterol diet induced hyperlipidemia. International Journal of PharmTech Research. 2010;2(4):2517–2521.
- [16] Kumar A, Sivashanmugam A, Umamaheswari M, Subhadradevi V, Jagannath P. Cholesterol Esterase Enzyme Inhibitory and Antioxidant Activities of Leaves of Camellia sinensis (L.) Kuntze. Using In Vitro Models. International Journal of Pharmaceutical Science Research. 2011;2(10):2675–2680.
- [17] Ojeda D, Ferrer E, Zamilpa A, Arellano A, Tortoriello J, Alvarez L. Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin and cyanidin-3-O-sambubiosides from Hibiscus sabdariffa. Journal of Ethnopharmacology. 2010;127:7–10.
- [18] Grundy S, Cleeman J, Merz C. Implication of recent clinical trials for the National Cholesterol Education Program Adult Panel on Detection, Evaluation and Treatment of high blood cholesterol in adults. JAMA. 2004;285:2486–2497.
- [19] Davidson M, Tooth P. Comparative effect of lipid lowering therapies. Prog Cardiovasc Dis. 2004;47:173–204.
- [20] Wang H, Ng T. Natural products with hypoglycaemic, hypotensive, hypocholesterlemic, antiathero-sclerotic and antithrombotic activities. Life Sci. 1999;65:2663–2677.
- [21] Schurr P, Schultz J, Parkinson T. Triton induced hyperlipidemia in rats as an animal model for screening of hypolipidemic drugs. Lipids. 1972;7:68–74.
- [22] Otway S, Robinson D. The effect of nonionic detergent (Triton WR 1339) on the removal of triglyceride fatty acids from the blood of the rats. J Physiol. 1967;19:309–319.
- [23] Pankaj G, Sanjay J. Evaluation of Antihyperlipidemic potential of Prosopis cineraria extract against high fat diet induced hyperlipidemia in laboratory rat. Pharmacologia. 2015;7:44–52.
- [24] Khanna A, Rizvi F, Chander R. Lipid lowering activity of Phyllanthus niruri in hyperlipemic rats. J Ethnopharmacology. 2002;82:19–22.
- [25] Brodt-Eppley J, White P, Jenkins S, Hui D. Plasma cholesterol esterase level is a determinant for an atherogenic lipoprotein profile in normolipidemic human subjects. BiochimBiophys Acta. 1995;1272:69–72.
- [26] Myers-Payne S, Hui D, Brockman H, Schroeder F. Cholesterol esterase: a cholesterol transfer protein. Biochem. 1995;34:3942–3947.
- [27] Nwaoguikpe R, Ujowundu C, Wesley B. Phytochemical and biochemical compositions of African Walnut (Tetracarpidium conophorum). J Pharm Biomed Sci. 2012;20(9):1–5.
- [28] Apeh V, Agu C, Ogugua V, Uzoegwu P, Anaduaka E, Rex T, et al. Effect of Cooking on Proximate, Phytochemical Constituents and Hematological Parameters of Tetracarpidium conophorum in Male Albino Rats. European Journal of Medicinal Plants. 2014;4(12):1388–139.
- [29] Elke A, Guus S, Yuguang L, Sergey M, Henri O, Fady Y. Proposed mechanisms of cholesterol lowering action of plant sterol. Eur J Lipid Sci Technol. 2003;105:171–185.
- [30] Ostlund R, Racette S, Okeke A, Stenson W. Phytosterol that are naturally present in commercial corn oil significantly reduce cholesterol absorption in human. Am J clin Nutr. 2002;75(6):1000–1004.
- [31] Heynekamp J, Hunsaker L, Vander J, Royer R, Decka M, Vander J. Isocoumarin-based inhibitors of pancreatic cholesterol esterase. Bioorganic & Medicinal Chemistry. 2008;16:5285–5294.
- [32] Kirana C, Rogers P, Bennett L, Abeywardena M, Patten G. Naturally derived micelles for rapid in vitro screening of potential cholesterol-lowering bioactives. J Agric Food Chem. 2005;53:4623–4627.